

WHAT IS CLAIMED IS:

1. A method for detecting autoantibodies to an approximately 64kD pancreatic  $\beta$ -cell autoantigen in sera, said method comprising exposing a serum sample to purified ligand for the autoantibodies and detecting specific interaction between the purified ligand and the autoantibodies.

2. A method as in claim 1, wherein the purified ligand is glutamic acid decarboxylase or a fragment thereof.

3. A method as in claim 2, wherein the glutamic acid decarboxylase is isolated from a natural source.

4. A method as in claim 2, wherein the glutamic acid decarboxylase is synthetic.

5. A method as in claim 2, wherein the glutamic acid decarboxylase is lower molecular weight CNS GAD.

6. A method as in claim 2, wherein the glutamic acid decarboxylase is pancreatic GAD.

7. A method as in claim 1, wherein the serum sample is combined with soluble, labelled autoantibodies so that labelled and unlabelled autoantibodies compete to form complexes with the purified ligand, whereby the amount of label bound in such complexes is inversely proportional to the concentration of autoantibodies initially present in the serum sample.



5

10

15

20

25

30

35

21. A method as in claim 15, wherein the autoantibodies are labelled with an enzyme and binding of ligand to the autoantibodies inhibits enzyme activity.

22. A method as in claim 20, wherein the purified glutamic acid decarboxylase is bound to a solid phase and the method further comprises removing the solid phase from the serum sample in order to separate labelled complexes from unbound, labelled autoantibodies.

23. A method as in claim 22, wherein the label present on the solid phase is measured.

24. A method as in claim 22, wherein the label remaining in the serum sample is measured.

25. A method as in claim 15, wherein the purified glutamic acid decarboxylase is bound to a solid phase and the method further comprises separating the solid phase to remove substantially all autoantibodies present in the sample and measuring the amount of autoantibodies removed, whereby the amount is directly proportional to the concentration of autoantibodies present in the sample.

26. A method as in claim 25, wherein the amount of autoantibodies is measured by exposing the solid phase to labelled reagent specific for the autoantibodies and determining the amount of bound labelled reagent.

27. A method as in claim 15, wherein the purified glutamic acid decarboxylase possesses native enzyme activity and the interaction is detected based on loss of enzyme activity.

28. A method for diagnosing insulin dependent diabetes mellitus and related conditions in a patient, said method comprising:

obtaining a serum sample from the patient;

assaying the sample to determine the presence of autoantibodies against glutamic acid decarboxylase, whereby presence of such antibodies is diagnostic of insulin dependent diabetes mellitus.

5

29. A method as in claim 28, wherein the assay is a radioimmunoassay employing radiolabelled glutamic acid decarboxylase bound to a solid phase.

10

30. A method as in claim 28, wherein the assay is an enzyme linked immunoadsorbent assay employing enzyme-labelled glutamic acid autoantibodies and glutamic acid decarboxylase bound to a solid phase.

15

31. A method for inhibiting the development of insulin dependent diabetes mellitus, said method comprising administering to a patient a preselected dosage of glutamic acid decarboxylase or a fragment thereof.

20

32. A method as in claim 31, wherein the glutamic acid decarboxylase or fragment thereof is coupled to an immunoglobulin or lymphoid cell from the patient being tested.

25

33. A method as in claim 32, wherein the glutamic acid decarboxylase or fragment thereof has been modified to decrease binding to an associated T-cell receptor while maintaining binding to the MHC, whereby the cellular immune response is inhibited.

30

34. A method as in claim 31, wherein the dosage of glutamic acid decarboxylase is selected to induce tolerance in the patient to the 64kD autoantigen associated with insulin dependent diabetes.

35

08838486 040757

~~SECRET~~

5

10

15

20

25

30

35

000000000000

43. A method for diagnosing or monitoring insulin dependent diabetes mellitus and related conditions in a patient, said method comprising:

obtaining a serum sample from the patient;  
detecting in the sample the presence of autoantibodies to lower molecular weight glutamic acid decarboxylase (GAD); and

detecting in the sample the presence of autoantibodies to higher molecular weight glutamic acid decarboxylase (GAD), wherein the presence of autoantibodies to at least one of the molecular weight forms of GAD indicates the onset or persistence of insulin dependent diabetes mellitus.

44. A method as in claim 43, wherein the autoantibodies to each molecular weight form of GAD are detected separately so that the presence of each form is known.

45. A method as in claim 44, wherein the presence of autoantibodies to each molecular weight form of GAD is separately determined by reaction with recombinantly produced GAD which is free from the other molecular weight form.

46. A method as in claim 43, wherein the autoantibodies to each molecular weight form of GAD are detected simultaneously so that the presence of neither form is individually determined.

47. A method as in claim 46, wherein the presence of the autoantibodies is determined by reaction with a mixture of both molecular weight forms of GAD.

48. A method as in claim 47, wherein the mixture is isolated from a source of native CNS GAD.

add B4  
add C5  
add 1.2  
add H1

0833486 0409